SYNTHESIS OF PORPHINE-LIKE SUBSTANCES

DURING CHEMICAL EVOLUTION

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INTRODUCTION

It is already a well established theory that chemical evolution, i.e. evolution on the molecular level, has preceded the appearance of the first living cell on the earth. Current concepts suggest that a relatively simple primitive atmosphere, consisting of methane, ammonia, water, and also probably some molecular hydrogen under influence of ultraviolet radiation, electrical discharges and high energy radiations has been transformed into more complicated compounds (1, 2, 3). It has been generally accepted that the temperature at the time of this transformation was less than 100°C. The raw material thus produced, accumulated in pools of water that condensed, forming an organic millieu, i.e. the nonliving mixture of carbon compounds which is generally considered as a prerequisite of the origin of life. Some estimates made by Urey (2) indicate that the primitive oceans might have contained as much as 10 per cent of dissolved organic compounds. The synthesis of these raw materials continued until a state of equilibrium was reached, in which the action of available energy sources produced as much of the raw material as it destroyed. In this period of time only relatively simple molecules could be formed because the more complicated and labile ones would be destroyed by the energetic ultraviolet radiation reaching the surface of the earth, unscreened by the ozone layer, which now protects the earth.

The above theories of Oparin (1) and Urey (2) have been subjected to rigid laboratory tests by Miller (4,5), Pavlovskaya and Pasynskii (6), Abelson (7), Oro (8), Hasselstrom (9), Paschke (10), and others (11, 12). This laboratory work has produced various products of biochemical importance, such as amino acids, aliphatic acids, polyhydroxy compounds, aldehydes and urea. Subjecting these simple molecules to further action of ultraviolet radiation or mild heating, produced a variety of complex organic molecules. Such . higher order reactions, for example, result in production of poly- peptides from amino acids, as demonstrated by Fox (13, 14), Akabori (15), Doty (16), and others (17), in actual laboratory experiments.

In the reviewed literature, most of the authors (1, 18, 19, 20) dealing with the problem of the origin of life, are convinced that at some stage of chemical evolution, porphyrins must have developed. However, until recently there was no laboratory evidence that porphyrins or porphine-like substances could be synthesized from precursors which were available during the period of chemical evolution on earth.

It has been shown in our previous paper (21), that porphine- like substances, specifically α, β, γ, δ -tetraphenylporphines, can be synthesized from simple precursors using γ-radiation for activation purposes.

This paper presents another observation having direct bearing on the formation of the organic millieu, namely the synthesis of porphine-like substances from simple precursors.

EXPERIMENTAL PROCEDURE

A system, consisting of 3 ml of freshly distilled pyrrole, 6 ml of benzaldehyde, and 4 ml of water, was placed in a 250 ml beaker, and then irradiated, at a distance of 25 cm with ultra-violet light, using a 100 Watt Hanovia Utility Model Lamp. The radiation transmitted through the filter was 45 per cent at 2500 A, 90 per cent at 3000 A and the output of the lamp was 1.5×10^{15} quanta per sec per cm^2 or 5×10^{16} quanta per sec per area of the mixture exposed toward radiation. The doses were measured by chemical actinometry, using an uranyl oxalate actinometer according to Forbes and Heidt (22). After exposure, the reddish-brown solution was diluted with approximately 20 ml of chloroform and washed with 20 ml of distilled water. After separation in a separatory funnel, the aqueous layer was discarded, and the chloroform layer was transferred into a beaker charged with approximately 20 g of anhydrous sodium sulfate to remove the excess of water. The sodium sulfate was then filtered off and discarded, while the filtrate was made up to 50 ml volume with chloroform and stored for the quan-titative determination of porphines.

The final product separation was accomplished by column chroma-tography, using activated alumina (F-20) and Florosil (60-100 mesh) as adsorbents and fresh chloroform as eluent. Two chromatographic treatments on alumina and three to four chromatographic separations on Florosil yielded pure product. The porphines appeared in

eluents and collections of eluent were continued until no Soret

band appeared in the effluent. The final spectrum of the product

was recorded on a Bausch and Lomb Spectronic 505 recording spectro-

photometer, using benzene as a solvent.

A typical spectrum of compound isolated from irradiated mixture

and the reference spectrum of the compound synthesized by the method

of Ball, et al. (23), are presented in Fig. 1. Both spectra are

identical with those obtained by Thomas et al. (24), and Dorough

et al. (25). For quantitative estimation of the yield, the molar .

extinction coefficient of 18.7×10^3 at 515 mu maximum was used, as

suggested by Thomas and Martell (24). Identity of the compound was

further established by the formation of a zinc chelate, according to

the procedure of Rothemund and Menotti (26), and Dorough et al. (25).

Both spectra, the reference zinc chelate and the chelate of the

compound isolated from the irradiated mixture are identical, as

shown in Fig. 2.

The same mixture of pyrrole, benzaldehyde and water when

placed in the dark without irradiation, also produced porphines.

The isolation and quantitative determination of porphines was

done by the same methods used for irradiated mixtures.

If, instead of water, pyridine was used as a solvent in the

system, and the mixture was then irradiated for two hours, no

porphine-like substances could be detected by previously used methods.

Aging of the mixture for ten days produced only traces of porphines.

Fig. 3 represents the total yields of porphines isolated
from the stored mixtures, plotted against time of storage. Each
line in this graph represents different irradiation periods.

DISCUSSION

Literature dealing with the origin of life, particularly the
role of porphines and porphyrins in life's origin have raised several
important questions. As yet, they are unanswered; the future can
and must answer them.

In our present study we have shown that porphine-like structures
can be synthesized in the presence of oxygen from precursors which
were available in the very earliest stage of chemical evolution.
Aldehydes were found in products of the action of electrical dis-
charges on the Urey atmosphere in Miller's experiment (4, 5). Pyrroles
and pyrrolidines were easily formed from ammonia, acetylene, and
other unsaturated hydrocarbons by simple catalysis or under influence
of ultraviolet radiation (1). In addition, Lichtin (27) found
pyrroles as the major product of the reaction of active nitrogen
with 1,3-butadienes.

The results of this study also indicate that the presence of
water, or rather the suspension of the organic matter in water,
increases the yield of porphine like substances considerably.
Oparin (1), and Haldane (3), have already indicated the possibility

of evolution of living matter from the pools rich in organic matter,
rather than on solid surfaces. The concentration of organic matter
could be quite high, if part of the water solvent evaporated, as the
case might be in lakes and lagoons.

The increase in the yield of porphine-like substances, on
standing, shows that a process of autocatalysis takes place. This
process, postulated by Calvin (19), now can be supported by
experimental evidence.

There is a general agreement among many authors that synthesis
of porphyrins is a necessary step for the origin of life. However,
the question of the time of the appearance of porphyrins is subject to
discussion. Gaffron (18), and Calvin (19), support the idea that
porphyrins were already active in the very earliest quasi-living
organic structures. Miller and Urey (5), consider this not a necessity
and suggest that porphyrins may have arisen during the evolution of
primitive organisms. Strughold and Ritter (28), consider pre-existing
stores of oxygen, produced by photochemical dissociation of water,
as a prerequisite for the formation and development of chlorophyll,
while the heme types of porphyrins were formed about 1 to 1.5
billion years later.

At the present time, it seems to be impossible to determine
at what stage of chemical evolution, porphine-like substances
developed. In the present living processes, porphine-like substances
find a great variety of uses, such as fermentation, respiration, and
photosynthesis. The metabolism of sulfates, iron, nitrogen, hydrogen,

and oxygen also require porphrins. In addition, an interesting
observation was made by Baltrop (19c) on the formation of pyro-
phosphates from phosphates in presence of ferrous protoporphyrins.
All these facts would suggest that porphyrins evolved prebiologically
in order to facilitate the evolution of seemingly living organic
structures up to the present state. This would place the appearance
of porphyrins in the oxygen free period of chemical evolution.

On the other hand, after the stores of simpler organic compounds
reached the saturation point, in the first stage of chemical evolution,
there was need for a reduction of the energy level of the source in
order to continue chemical evolution to a more complicated nature.
Photolysis of water and the formation of an ozone layer cut out ultra-
violet radiation completely, while visible light itself did not provide
enough energy for chemical synthesis. With the aid of porphyrins and
especially their metal chelates, chemical transformations such as
hydrogen transfer or oxidation could occur.

Simultaneously with this event, hydrogen peroxide appeared. If
it remained in contact with organic substances, the results would be
fatal. Therefore, in order to continue chemical evolution, it was
necessary that a mechanism be developed for the destruction of vast
amounts of hydrogen peroxide. Calvin (19d), has already pointed out
that incorporation of ferric iron into a heme-type molecule would
increase the catalytic activity of iron by a factor of 10^3 and the
addition of certain protein arrangements by an additional factor of
10^5.

The last two arguments indicate that with the transition of

the reductive atmosphere into oxidative, an immediate need was created for the porphine-like substances. This need served as an evolutionary selection pressure for the synthesis of porphine-like substances.
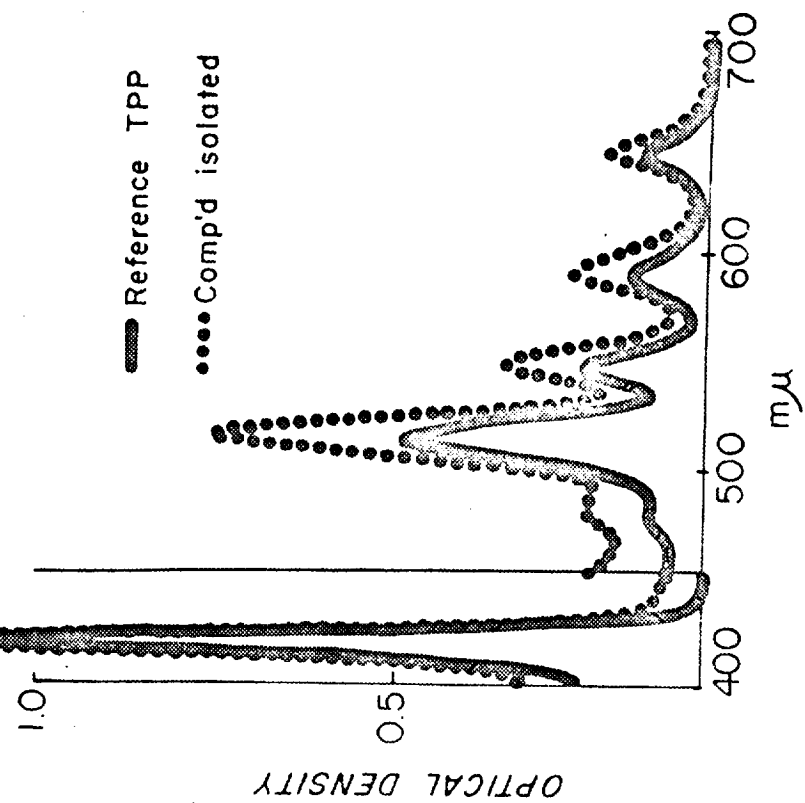
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SPECTRA IN BENZENE SOLUTION

■■■ Reference TPP
●●●● Comp'd isolated

OPTICAL DENSITY

mμ

Zn-Chelate SPECTRA in BENZENE

—— Reference Zn-Chelate
- - - Compound isolated

OPTICAL DENSITY

mμ

YIELD OF TPP versus LENGTH OF STORAGE

IRRADIATION CONDITIONS:

0 HRS IN PYRIDINE
0 HRS AQUEOUS SUSPENSION
1 HR AQUEOUS SUSPENSION
2 HRS AQUEOUS SUSPENSION
3 HRS AQUEOUS SUSPENSION